

EVI27 GENE SEQUENCES AND PROTEIN ENCODED THEREBY

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BACKGROUND OF THE INVENTION

Cross-Reference to Related Application

This non-provisional patent application claims benefit of provisional patent application U.S. Serial Number 60/180,374, filed February 4, 2000, now abandoned.

Federal Funding Legend

This invention was produced in part using funds obtained through a grant from the National Cancer Institute. Consequently, the federal government has certain rights in this invention.

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Field of the Invention

The present invention relates generally to the field of molecular biology. More specifically, the present invention relates to the cloning and characterization of a murine and human gene 5 that encodes a novel protein with homology to the IL-17 receptor.

Description of the Related Art

Retroviral insertional mutagenesis in BXH2 and AKXD recombinant inbred (RI) mice induces a high incidence of myeloid leukemia and the proviral integration sites in the leukemias provide powerful genetic tags for disease gene identification (Bedigian et al., 1984; Gilbert et al., 1993). During the past several years, a number of disease genes have been identified in these leukemias by proviral tagging. These disease genes include a tumor suppressor gene, 15 *neurofibromatosis type 1 (Nf1)*; a gene with homology to the lymphoid-restricted type II membrane protein Jaw1, *Mrv integration site 1 (Mrvil)*; a gene encoding a hematopoietic cell growth and differentiation factor, *myeloblastosis oncogene (Myb)*; three homeobox genes, *homeobox A7 (Hoxa7)*, *homeobox A9 (Hoxa9)*, 20 and *myeloid ecotropic viral integration site 1 (Meis1)*; a zinc-finger

protein (*Evi1*); and a gene with homology to the *ubiquitin-specific protease 8* (*Usp8*) oncogene and to genes encoding various cell cycle regulatory proteins, *ecotropic viral integration site 5* (*Evi5*) (Buchberg et al., 1990, Viskochil et al., 1990, Shaughnessy et al.,
5 1999; Copeland and Jenkins, 1999, Nakamura et al., 1996a, Morishita et al., 1988, Liao, et al., 1997). Four of the genes are proven or suspected human disease genes: *EVII*, *NFI* and *HOXA9* are causally associated with myeloid leukemia and *EVI5* with stage
10 4S neuroblastoma (Ogawa et al., 1996, Copeland and Jenkins, 1999, Nakamura et al., 1996b, Roberts et al. 1998), validating the usefulness of this approach for human disease gene identification.

Although proviral tagging has identified many disease genes, it is apparent that several more genes remain to be cloned. This is suggested by the fact only 45% of BXH2 leukemias contain a
15 virally induced mutation in one of the genes identified so far. Disease genes for 55% of BXH2 leukemias remain to be identified. The same is true for human acute myeloid leukemias (AMLS) where the 11 different chromosomal translocations and inversions cloned to date are found in only 45% of acute myeloid leukemias (Look,
20 1997). Disease genes for 55% of acute myeloid leukemias remain to

be identified. Ultimately, it should be possible to use proviral tagging to do a saturation screen for BXH2 disease genes. The expectation is that some of these genes will represent human acute myeloid leukemias genes that are not easy to clone because they are infrequently involved in human disease or are not marked by a cytologically detectable rearrangement. Given the large number of genes that may remain to be identified, this task could be difficult using conventional proviral tagging approaches, which rely on cloning leukemia-specific proviral integration sites into bacteriophage lambda.

PROVIRAL TAGGING

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With this potential problem in mind, an inverse PCR (IPCR) method for proviral tagging was developed that makes use of automated DNA sequencing and the genetic tools provided by the Mouse Genome Project, which greatly increases the throughput of proviral tagging for disease gene identification. More than 400 proviral integration sites from BXH2 myeloid leukemias (and AKXD T- and B-cell leukemias) were cloned and characterized using this inverse PCR method (Li et al., 1999), which lead to the identification of more than 90 new candidate leukemia disease genes (Li et al., 1999). Nineteen new common integration sites (sites that are

targets of viral integration in more than one leukemia) were also identified in these studies and BLAST search and/or chromosome mapping identified candidate disease genes for 12 of these common sites (Li et al., 1999).

5 One common integration site identified by the inverse PCR is *Evi27* (Li et al., 1999). While BLAST searches did not identify a candidate disease gene for *Evi27*, chromosome mapping studies showed that *Evi27* maps to mouse chromosome 14 in a region of human 3p21 homology (Copeland et al., 1993). This result is 10 interesting because treatment-related 3p21 breaks are often observed in myelodysplastic syndrome (MDS) and AML patients (Shi et al., 1996) and 3p21 is the most frequently deleted region seen in chronic myeloid leukemia (CML) (Johansson et al., 1997). *Evi27* may be, therefore, an important human disease gene.

15 The prior art is deficient in lack of the characterization a novel cytokine receptor-related gene whose expression is upregulated by viral integration at *Evi27*. The present invention fulfills this long-standing need and desire in the art.

SUMMARY OF THE INVENTION

The present invention describes the cloning and sequences of a novel IL-17 receptor-related gene in human and mouse whose expression is upregulated by viral integration in a murine acute myeloid leukemia. Gene transcription and protein expression were examined by northern blot analysis, western blot analysis and immunohistochemical staining. The gene disclosed herein may facilitate myeloid cell transformation and be involved in human disease.

BRIEF DESCRIPTION OF THE DRAWINGS

The appended drawings have been included herein so that the above-recited features, advantages and objects of the invention will become clear and can be understood in detail. These drawings form a part of the specification. It is to be noted, however, that the appended drawings illustrate preferred

embodiments of the invention and should not be considered to limit the scope of the invention.

Figure 1 shows a partial long-range restriction map of the *Evi27* locus. Bacteriophage λ and P1 clones used for gene localization and exon trapping are shown above the map. The location of the 5' end and transcriptional orientation of the *Evi27* and *Cdhd* genes are noted by arrows above the map. The location of the exon with homology to the yeast *ARP8* gene is also noted. The transcriptional orientation of this gene has not been determined. The position and transcriptional orientation of the proviruses in the B160 and 15-38374 leukemias are indicated below the map. The cluster of rare cutting restriction enzymes surrounding the proviral integration sites is also noted. The 14kb *Bam*HI fragment cloned from leukemia B160 is presented below the map. The viral sequences and viral long terminal repeat-(LTR) is noted by a thick line and black box, respectively. The position of the 5' end of *Evi27* with respect the viral LTR is noted. Restriction enzymes: B, *Bam*HI; Bs, *Bss*HIII; C, *Clai*; E, *Eag*I; M, *Mlu*I, N, *Nae*I; Nr, *Nru*I, S, *Sac*II; Sa, *Sali*I;

Figure 2 shows Northern blot analyses of *Evi27* expression in murine tissues and cancer cell lines. Multiple tissue

Northern blots of normal adult (**Figure 2A**), BXH2 leukemic cell lines (**Figure 2B**), and various hematopoietic cell lines (**Figure 2C**): M1, myeloid leukemia; WEHI3B, monocyte; HYB. 548, B-cell hybridoma; WEHI231, preB-cell lymphoma; R1.1, lymphocytic thymoma; EL4, thymoma, P815, mastocytoma. The blots were also hybridized with a GAPDH or β -actin probe to control for RNA loading. The size in kilobases (kb) of molecular weight markers is show on the left of each panel. To the right of each panel are the sizes of the Evi27 transcripts observed

Figure 3 shows Northern blot analyses of *EVI27* expression in human tissues and cancer cell lines. Multiple tissue Northern blots of normal adult (**Figure 3A**), immune tissues (**Figure 3B**) and cancer cell lines (**Figure 3C**) hybridized with an EST specific for the human *EVI27* gene. The human cancer cell lines are HL60, promyelocytic leukemia; HeLa, cervical carcinoma; K562, chronic myelogenous leukemia; MOLT4, T-lymphoblastic leukemia; Raji, Burkitt's lymphoma; SW480, colon adenocarcinoma; A549, lung carcinoma; G361, melanoma. The blots were also hybridized with a β -actin probe to control for RNA loading. The size in kb of molecular weight markers is show on the left of each panel.

Figure 4 shows an alignment of mouse and human *Evi27* proteins. The putative signal peptide, transmembrane domain, and peptide used to generate polyclonal antisera are indicated by a black line over the sequence. Conserved protein motifs are indicated by a double line. Conserved amino acids are boxed with amino acid identities noted in bold. Normal typeface letters represents conservative amino acid changes. Gaps created in the sequences to optimize alignments are indicated by a dash in the sequence string. Abbreviations: N-GLY, N-linked glycosylation; GSK, kinase phosphorylation site.

Figure 5 shows the *Evi27* gene structure. The gene consists of 11 exons with the first and last exons containing untranslated regions. The transmembrane domain is located in exon 10.

Figure 6 shows an alignment of the human EVI27 and IL-17R proteins. Conserved amino acids are boxed with amino acid identities noted in bold. Gaps created in the sequences to optimize alignments are represented by dashes. Amino acid positions are indicated to the right and left of the sequence.

Figure 7 shows the chromosome mapping of the human

EVI27 gene. **Figure 7A** fluorescence *in situ* hybridization of a PAC clone specific for *EVI27 gene* to normal human metaphase chromosomes. Arrows indicate localization of the probe. **Figure 7B** shows G-banding of the same metaphase spread in (**Figure 7A**) following destaining of FISH reagents. The probe is localized to band 3p21.

Figure 8 shows immunofluorescence and Northern blot analysis of *Evi27* expression in murine cell line. The 2.7 kb cDNA coding for the 55 kD isoform of the membrane bound form of the murine *Evi27* gene was cloned into a eukaryotic expression plasmid vector and transfected into the murine myeloid leukemia cell line 32D. A cell line was established by limiting dilution and called 32DEvi27A. **Figure 8A** shows a Northern blot hybridization of poly-A mRNA from 32D and 32D/Evi27A stable transfectants. Note the abundant expression of the transgene in the transfectant and the absence of expression in the parental line. The blot was also hybridized with a β -actin probe to control for RNA loading. The size in kb of molecular weight markers is show on the left of each panel.

Figure 8B shows Western bolt analysis of same cell lines with affinity purified anti-Evi27 antisera. The transfectant shows

overexpression of a 55 kD protein as expected from predicted mRNA translation of the 2.7 kb Evi27 cDNA. Markers in kD are to the left. **Figure 8C** shows immunofluorescence staining of the Evi27 protein (red) in the myeloid cell line 32D (top panel) and 32D 5 cells transfected with an *Evi27* cDNA expression construct (lower panel). Note the light staining in the parental line and abundant staining in the transfectant (left panels). Nuclei are stained blue with DAPI. Cells were also stained with Evi27 antibody preincubated with Evi27 peptide (right panels). Note that no red staining is evident, demonstrating specificity of the antisera. **Figure 8D** shows cell surface expression of Evi27 by flow activated cell sorting analysis (red: anti-Evi27; blue: anti-Evi27+peptide; green: IgG control).

Figure 9 shows cell surface expression and capping of 15 Evi27 on murine T cell lymphoma EL-4. The left panel shows immunochemistry of the EL-4 cells with the anti-Evi27 antibody before and after pre-treatment with Evi27 peptide. Right panel shows FACS analysis with increasing amount of anti-Evi27 antibody. The capping of Evi27 possibly indicates polydimerization and 20 receptor activation of Evi27.

Figure 10 shows subcellular localization of Evi27 in myeloid leukemia B187 and B190. The left panel shows immunochemistry of the B187 and B190 cells with the anti-Evi27 antibody before and after pre-treatment with Evi27 peptide. B187 5 cells show diffuse speckled staining in the cytoplasm, whereas B190 cells shows a capping pattern similar to the EL-4 cells. The signals could be competed away by preincubation with Evi27 peptide. Right panel shows western blot analysis of same cell lines with anti-Evi27. Both cell lines express multiple isoforms of Evi27, and B187 cells express more of the 55 kD isoform. The bands could be competed away by preincubation with Evi27 peptide.

Figure 11 shows abundant Evi27 protein was found in the cytoplasm, but not on the cell surface, in the myeloid leukemia B160. The left panel shows immunochemistry of the B160 cells with 15 the anti-Evi27 antibody before and after pre-treatment with Evi27 peptide. Evi27 is expressed in the cytoplasm at high concentration. Right panel shows FACs analysis with anti-Evi27, indicating Evi27 is not expressed on the cell surface (red: anti-Evi27; black: IgG control).

20 **Figure 12** shows Western blot analysis of Evi27 protein

expression in BXH2 leukemia cell lines, EL4 and R1.1 T-cell lines, and the WEHI231 B-cell line. 10 µg of protein from cell lysates was run in tandem and hybridized with Evi27 antibody either without (Figure 12A) or with (Figure 12B) preincubation with Evi27 peptide. Note the absence or reduction of specific bands in panel (Figure 12B) compared to (Figure 12A). Molecular weight standards in kD are indicated to the left. The Evi27 isoforms and sizes are indicated to the right of panel A.

Figure 13 shows western blot analysis of Evi27 expression in human cell lines. SW480 and SW620 are colon adenocarcinoma; MEG01, megakaryocytic leukemia; THP-1, monocytic leukemia; K562, chronic myelogenous leukemia; HL-60, promyelocytic leukemia; U266, ARP1 and 8226, multiple myeloma; HeLa, cervical carcinoma; HEL, erythrocytic leukemia. 55 kD and 30 kD are major bands recognized, thus the antibody recognizes both human Evi27 protein isoforms. The bands could be competed away by preincubation of Evi27 antisera with Evi27 peptide as shown in the right panel. MEG01, K562 and HEL cell lines express the highest levels of the Evi27 protein.

Figure 14 shows surface expression of Evi27 on human hematopoietic cell lines. Left panel shows FACS analysis of Evi27 expression on the surface of various human cell lines (red: affinity purified anti-Evi27 antisera; black: IgG control). Evi27 was expressed on THP-1 and K562 cells but not the HL-60 cells. Right panel shows western blot analysis of same cell lines with anti-Evi27. As expected from the FACS data, THP-1 cells express more Evi27 protein than K562 cells, which in turn express more Evi27 than HL-60 cells. It is also shown that K562 cells produce more of the 30 kD soluble form of the Evi27 protein.

DETAILED DESCRIPTION OF THE INVENTION

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Evi27 is a common site of retroviral integration in BXH2 murine myeloid leukemias. The present invention shows that integration at Evi27 occurs in a CpG island ~6 kb upstream from a novel gene (designated Evi27) with homology to the *IL17 receptor* (*Il17r*) and that proviral integrations result in increased Evi27

expression. The human *EVI27* homologue was also cloned and mapped to chromosome 3p21. Multiple *Evi27* isoforms were detected at the RNA and protein level in both human and mouse, indicating that *Evi27* expression is complex. Some of the isoforms
5 are shown to likely represent secreted soluble forms of the protein produced by intron incorporation or by proteolytic cleavage. In the mouse, highest *Evi27* expression occurs in liver and testes with lower expression in kidney and lung. In humans, *EVI27* is expressed at high levels in the kidney, with moderate levels in the liver, brain,
10 and pancreas. Within hematopoietic cells, *Evi27* expression is restricted. Northern and Western analysis showed that *Evi27* is expressed in selected T-cell, B-cell and myeloid cell lines. These results suggest that *Evi27* expression is tightly regulated during hematopoietic differentiation. Collectively, these studies identify a
15 new member of the cytokine receptor family whose increased and
uncoordinated expression [may lead to myeloid leukemia by altering]
Evi27's normal ability to control the growth and/or differentiation
of hematopoietic cells.

In accordance with the present invention, there may be
20 employed conventional molecular biology, microbiology, and

recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook, Fritsch & Maniatis, "Molecular Cloning: A Laboratory Manual (2nd Ed.)", (1989); "DNA Cloning: A Practical Approach," Volumes I and II (D.N. Glover ed. 1985); "Oligonucleotide Synthesis" (M.J. Gait ed. 1984); "Nucleic Acid Hybridization" [B.D. Hames & S.J. Higgins eds. (1985)]; "Transcription and Translation" [B.D. Hames & S.J. Higgins eds. (1984)]; "Animal Cell Culture" [R.I. Freshney, ed. (1986)]; "Immobilized Cells And Enzymes" [IRL Press, (1986)]; B. Perbal, "A Practical Guide To Molecular Cloning" (1984). Therefore, if appearing herein, the following terms shall have the definitions set out below.

A "DNA molecule" refers to the polymeric form of deoxyribonucleotides (adenine, guanine, thymine, or cytosine) in its either single stranded form or a double-stranded helix. This term refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear DNA molecules (e.g., restriction fragments), viruses, plasmids, and chromosomes. DNA structures are discussed herein according to

the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of DNA (*i.e.*, the strand having a sequence homologous to the mRNA). The term "peptide" or "polypeptide" is defined as several (*i.e.*, multiple) amino acids attached together.

A "vector" is a replicon, such as plasmid, phage or cosmid, to which another DNA segment may be attached so as to bring about the replication of the attached segment. A "replicon" is any genetic element (*e.g.*, plasmid, chromosome, virus) that functions as an autonomous unit of DNA replication *in vivo*; *i.e.*, capable of replication under its own control. An "origin of replication" refers to those DNA sequences that participate in or direct DNA synthesis. An "expression control sequence" is a DNA sequence that controls and regulates the transcription and translation of another DNA sequence. A coding sequence is "operably linked" and "under the control" of transcriptional and translational control sequences in a cell when RNA polymerase transcribes the coding sequence into mRNA, which is then translated into the protein encoded by the coding sequence.

In general, expression vectors containing promoter

sequences which facilitate the efficient transcription and translation of the inserted DNA fragment are used in connection with a particular host. The expression vector typically contains an origin of replication, promoter(s), terminator(s), as well as specific genes 5 which are capable of providing phenotypic selection in transformed cells (*i.e.*, selectable markers). The transformed hosts can be fermented and cultured according to means known in the art to achieve optimal cell growth.

A DNA "coding sequence" is a double-stranded DNA 10 sequence which is transcribed and translated into a polypeptide *in vivo* when placed under the control of appropriate regulatory sequences. Specifically as used herein, "DNA coding for a protein" means DNA sequences which produce a particular primary amino acid sequence. The boundaries of the coding sequence are 15 determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxyl) terminus. A coding sequence can include, but is not limited to, prokaryotic sequences, a cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (*e.g.*, mammalian) organisms and even synthetic DNA 20 sequences. A polyadenylation signal and transcription termination

sequence will usually be located 3' of the coding sequence. A "cDNA" is defined as copy DNA or complementary DNA and is a product of a reverse transcription reaction from an mRNA transcript. An "exon" is an expressed sequence transcribed from 5 the gene locus, whereas an "intron" is a non-expressed sequence that is from the gene locus.

Transcriptional and translational control sequences are DNA regulatory sequences, such as promoters, enhancers, polyadenylation signals, terminators, and the like, that provide for 10 the expression of a coding sequence in a host cell. A "cis-element" is a nucleotide sequence, also termed a "consensus sequence" or "motif", that interacts with proteins that upregulate or downregulate expression of a specific gene locus. A "signal sequence" can also be included with the coding sequence. This sequence encodes a signal 15 peptide, N-terminal to the polypeptide, that communicates to the host cell and directs the polypeptide to the appropriate cellular location. Signal sequences can be found associated with a variety of proteins native to prokaryotes and eukaryotes.

A "promoter sequence" is a DNA regulatory region 20 capable of binding RNA polymerase in a cell and initiating

transcription of a downstream (3' direction) coding sequence.

Specifically as used herein, the term "promoter(s)" means regulatory DNA sequences that control transcription of the cDNA.

For purposes of defining the present invention, a minimal promoter

5 sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site, as well as protein binding domains (consensus sequences) responsible for the binding 10 of RNA polymerase. Eukaryotic promoters often, but not always, contain "TATA" boxes and "CAT" boxes. Prokaryotic promoters contain the -10 and -35 consensus sequences, and additionally, ribosomal binding Shine-Dalgarno sequences. As used herein,

15 "promoter" may also refer to an intact regulatory sequence directing transcription (and subsequent translation) of a coding sequence, and may include any or all of the above-mentioned transcriptional and translational control sequences.

The term "oligonucleotide" is defined as a molecule
20 comprised of two or more deoxyribonucleotides, preferably more

than three. Its exact size will depend upon many factors which, in turn, depend upon the ultimate function and use of the oligonucleotide. The term "primer" as used herein refers to an oligonucleotide, whether occurring naturally as in a purified restriction digest or produced synthetically, which is capable of acting as a point of initiation of synthesis when placed under conditions in which synthesis of a primer extension product, which is complementary to a nucleic acid strand, is induced, *i.e.*, in the presence of nucleotides and a polymerizing agent, such as a DNA polymerase, and at a suitable temperature and pH. The primer may be either single-stranded or double-stranded and must be sufficiently long to prime the synthesis of the desired extension product in the presence of the polymerizing agent. The exact length of the primer will depend upon many factors, including temperature, source of primer and use the method. For example, for diagnostic applications, depending on the complexity of the target sequence, the oligonucleotide primer typically contains 15-25 or more nucleotides, although it may contain fewer nucleotides.

"Recombinant DNA technology" refers to techniques for uniting two heterologous DNA molecules, usually as a result of *in*

vitro ligation of DNAs from different organisms. Recombinant DNA molecules are commonly produced by experiments in genetic engineering. Synonymous terms include "gene splicing", "molecular cloning" and "genetic engineering". The product of these 5 manipulations results in a "recombinant" or "recombinant molecule".

A cell has been "transformed" or "transfected" with exogenous or heterologous DNA when such DNA has been introduced into the cell. Specifically as used herein, the term 10 "transformation" or "transfection" means incorporation permitting expression of heterologous DNA sequences by a cell. The transforming DNA may or may not be integrated (covalently linked) into the genome of the cell. In prokaryotes, yeast, and mammalian cells, the transforming DNA may be maintained on an episomal 15 element such as a vector or plasmid. With respect to eukaryotic cells, a stably transformed cell is one in which the transforming DNA has become integrated into a chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eukaryotic cell to establish cell 20 lines or clones comprised of a population of daughter cells

containing the transforming DNA. A "clone" is a population of cells derived from a single cell or ancestor by mitosis. A "cell line" is a clone of a primary cell that is capable of stable growth *in vitro* for many generations. An organism, such as a plant or animal, that has 5 been transformed with exogenous DNA is termed "transgenic".

As used herein, the term "host" is meant to include not only prokaryotes, but also eukaryotes such as yeast, plant and animal cells. Specifically as used herein, the term "host(s)" means any cell that will allow or direct expression. Specifically as used 10 herein, "chimeric cell" means a cell whose DNA has been altered compared to a normal cell of the same organism. A recombinant DNA molecule or gene can be used to transform a host using any of the techniques commonly known to those of ordinary skill in the art. Prokaryotic hosts may include *E. coli*, *S. typhimurium*, 15 *Serratia marcescens* and *Bacillus subtilis*. Eukaryotic hosts include yeasts such as *Pichia pastoris*, mammalian cells and insect cells, and plant cells, such as *Arabidopsis thaliana* and *Tobaccum nicotiana*.

As used herein, "fragment," as applied to a polypeptide, will ordinarily be at least 10 amino acid residues, more typically at 20 least 20 residues, and preferably at least 30 (*e.g.*, 50) residues in

length, but less than the entire, intact sequence. Fragments can be generated by methods known to those skilled in the art, e.g., by enzymatic digestion of naturally occurring or recombinant protein, by recombinant DNA techniques using an expression vector that 5 encodes a defined fragment, or by chemical synthesis. Purified fragments or antigenic fragments can be used to generate antibodies employing standard protocols known to those skilled in the art. As used herein, "functional fragment" is meant to encompass those peptide fragments retaining biological activity of Evi27.

10 Due to the redundancy of the DNA code, there are millions of DNA sequences that would produce the same amino acid sequence when expressed. Given an amino acid sequence, one can substitute into the natural DNA sequence alternative codons for the desired amino acids to produce an alternative DNA sequence also 15 coding for the novel protein. One may find that particular chimeric cells of a particular expression method favor particular mRNA codons for a particular amino acid. Altering the human DNA sequence to increase the frequency of favored codons may improve the expression efficacy in a chimeric cell, thus improving the 20 efficacy of the expression process. The sequences may be derived

by substitution of redundant codons for the amino acid sequences and splicing the substituted sequences into the natural gene by routine methods well known in the art. Those skilled in the art will recognize that many variations are possible in substituting 5 conserved amino acids in the protein sequence which will produce variations in sequence without seriously changing the biological activity of the protein. It is impractical to attempt to list all the millions of DNA sequences that may code for the claimed sequence. However, the invention comprises the novel protein, its novel amino 10 acid sequence, and all DNA sequences natural or synthetic coding for the novel amino acid sequence.

These substitution analogs may be constructed in the following manner: Table 1 lists the alternative codons that code for the 20 common amino acids. DNA sequence substitution analogs 15 that also code for human can be constructed by choosing alternate codons from Table 1 to alter the DNA sequence between a pair of restriction enzyme cleavage sites, as are well known in the art. Alternative codons are assembled into a synthetic oligonucleotide by conventional methods and the synthetic oligo is substituted into the 20 endonuclease treated DNA by the methods described in "Molecular

Cloning. A Laboratory Manual", 2d Edition, Cold Spring Harbor Laboratory Press (1989), to produce a substitution analog. Other methods generally known to those skilled in the art can also be employed to obtain substitution analogs of DNA sequences.

5 The alteration of the DNA by cleavage and codon substitution may be repeated to substitute substantial portions of the original DNA sequence with alternative codons without altering the protein amino acid sequence. Alteration of a DNA sequence which produces no change in the protein expressed by the DNA sequence might, for example, be conducted to increase protein expression in a particular host cell by increasing the occurrence of codons that correspond to amino acid tRNAs found in higher concentration in the host cell. Such altered DNA sequences for substitution analogs can be easily produced by those of ordinary skill in the art following the method set out above, or other alternative techniques for altering the DNA sequence while obtaining the same protein on expression. Substitution analogs can be obtained by substitution of oligonucleotides at restriction cleavage sites as described above, or by other equivalent methods that

10 change the codons while preserving the amino acid sequence of the

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expressed protein.

TABLE 1

		<u>SYMBOL</u>	<u>AMINO ACID</u>	<u>CODON USAGE</u>
		<u>1-Letter</u>	<u>3-Letter</u>	
5		A	Ala	Alanine GCT, GCC, GCA, GCG
		C	Cys	Cysteine TGT, TGC
		D	Asp	Aspartic acid GAT, GAC
		E	Glu	Glutamic acid GAA, GAG
10		F	Phe	Phenylalanine TTT, TTC
		G	Gly	Glycine GGT, GGC, GGA, GGG
		H	His	Histidine CAT, CAC
		I	Ile	Isoleucine ATT, ATC, ATA
		K	Lys	Lysine AAA, AAG
15		L	Leu	Leucine TTA, TTG, CTT, CTC, CTA, CTG
		M	Met	Methionine ATG
		N	Asn	Asparagine AAT, AAC
		P	Pro	Proline CCT, CCC, CCA, CCG
20		Q	Gln	Glutamine CAA, CAG
		R	Arg	Arginine CGT, CGC, CGA, CGG, AGA,
	AGG	S	Ser	Serine TCT, TCC, TCA, TCG, AGT,
	AGC			
25	T	Thr	Threonine ACT, ACC, ACA, ACG	
	V	Val	Valine GTT, GTC, GTG, GTG	
	W	Trp	Tryptophan TTG	
	Y	Tyr	Tyrosine TAT, TAC	

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As described herein, a standard Northern blot assay can be used to ascertain the relative amounts of mRNA in a cell or tissue in accordance with conventional Northern hybridization techniques

known to those persons of ordinary skill in the art. Alternatively, a standard Southern blot assay may be used to confirm the presence, the copy number and/or the position of a gene in accordance with conventional Southern hybridization techniques known to those of
5 ordinary skill in the art. Both the Northern blot and Southern blot use a hybridization probe, (e.g., radiolabelled full-length or partial cDNA) of at least 20 (preferably at least 30, more preferably at least 50, and most preferably at least 100) consecutive nucleotides in length. The DNA hybridization probe can be labelled by any of the
10 many different methods known to those skilled in this art.

The labels most commonly employed for these studies are radioactive elements, enzymes, chemicals which fluoresce when exposed to ultraviolet light, and others. A number of fluorescent materials are known and can be utilized as labels. These include,
15 for example, fluorescein, rhodamine, auramine, Texas Red, AMCA Blue and Lucifer Yellow. Proteins can also be labeled with a radioactive element or with an enzyme. The radioactive label can be detected by any of the currently available counting procedures. The preferred isotope may be selected from ^3H , ^{14}C , ^{32}P , ^{35}S , ^{36}Cl , ^{51}Cr ,
20 ^{57}Co , ^{58}Co , ^{59}Fe , ^{90}Y , ^{125}I , ^{131}I , and ^{186}Re .

Enzyme labels are likewise useful, and can be detected by any of the presently utilized colorimetric, spectrophotometric, fluorospectrophotometric, amperometric or gasometric techniques.

The enzyme is conjugated to the selected particle by reaction with bridging molecules such as carbodiimides, diisocyanates, glutaraldehyde and the like. Many enzymes which can be used in these procedures are known and can be utilized. The preferred are peroxidase, β -glucuronidase, β -D-glucosidase, β -D-galactosidase, urease, glucose oxidase plus peroxidase and alkaline phosphatase.

U.S. Patent Nos. 3,654,090, 3,850,752, and 4,016,043 are referred to by way of example for their disclosure of alternate labeling material and methods.

The present invention is directed to an isolated nucleic acid molecule encoding an IL-17 receptor-related protein selected from the group consisting of: (a) an isolated nucleic acid molecule of SEQ ID NO: 1, 2, 3 or 4 which encodes an IL-17 receptor-related protein; (b) an isolated nucleic acid molecule which is complimentary and hybridizes to the nucleic acid molecules of (a); and (c) isolated nucleic acid molecule differing from the isolated nucleic acid molecules of (a) or (b) in codon sequence due to the

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degeneracy of the genetic code, and which encodes an IL-17 receptor-related protein.

In one embodiment, there is provided a fragment of one of the nucleic acid molecules listed above that is at least 10 bases long and which will selectively hybridize to nucleic acid molecule encoding an IL-17 receptor-related protein. Preferably, the nucleic acid molecule may be used as an anti-sense molecule to inhibit the expression of an IL-17 receptor-related protein, for chromosomal mapping or mutation analysis of gene encoding an IL-17 receptor-related protein.

The present invention is also directed to a genomic DNA encoding an IL-17 receptor-related protein, wherein said genomic DNA hybridizes to the nucleic acid molecules described above.

The present invention is also directed to vector comprising a nucleic acid molecule of SEQ ID NO: 1, 2, 3 or 4 and cells transfected with the vector. Representative cells include bacterial cells, mammalian cells, plant cells and insect cells.

The present invention is also directed to an IL-17 receptor-related protein, or a peptide derived thereof, encoded by nucleic acid molecule of SEQ ID No. 3 or 4, wherein said protein is

about 24, 33, 56, 47, 75, 127, and 150 kD in size as detected by western blot analysis in BXH2 leukemia cell. The present invention is further directed to an IL-17 receptor-related protein, or a peptide derived thereof, encoded by the nucleic acid molecules disclosed 5 herein, wherein said protein has the amino acid sequence of SEQ. ID NO: 5, 6, 7 or 8. Preferably, the peptide is at least 4 amino acids long. The peptides can be used to generate anti-Evi27 antibody, and a person having ordinary skill in the art would be readily able to prepare an antibody that binds specifically to any of these proteins 10 or peptides.

The present invention is also directed to a method of stimulating the secretion of cytokines from a cell, comprising the step of binding a ligand to the IL-17 receptor-related protein, Evi27. In general, Evi27 stimulation can lead to the secretion of IL-1, IL-8 15 and TNF- α from hematopoietic cells, leukemia cells and kidney cells.

The present invention is also directed to a method of modulating the expression and activity of an IL-17 receptor-related protein, Evi27, comprising the step of contacting a molecule to a cell, wherein the binding of said molecule to Evi27 mRNA or protein 20 results in increased or decreased expression and activity of the

Evi27 protein. Preferably, the molecule can be anti-sense oligonucleotides, small molecule that binds to Evi27, modified IL-17E or soluble form of the Evi27 receptor.

The following examples are given for the purpose of
5 illustrating various embodiments of the invention and are not meant
to limit the present invention in any fashion:

EXAMPLE 1

BXH2 Mice and Leukemic Cell Lines

The BXH2 recombinant inbred strain was obtained from the Jackson Laboratory (Bar Harbor, ME) and maintained at the NCI-Frederick Cancer Research and Development Center. BXH2 leukemia
15 cell lines have been previously described (Largaespada et al., 1995).

Other cell lines were purchased from the ATCC and grown in either RPMI 1640 or DMEM supplemented with 2mM glutamine, 4.5 g/L glucose, Pen-Strep, and 10% fetal bovine serum (Atlanta Biologicals, Atlanta GA).

EXAMPLE 2

DNA Extraction and Southern Blot Hybridization

High molecular weight genomic DNAs were extracted
5 from frozen normal tissues and leukemic spleens and lymph nodes
as previously described (Jenkins et al., 1982). Bacteriophage and
plasmid DNAs were purified using standard procedures (Sambrook
et al., 1987). Restriction enzyme digestions, agarose gel
electrophoresis, Southern blot transfers, hybridizations, and washes
10 were performed as previously described (Sambrook et al., 1987).

EXAMPLE 3

Genomic Cloning

15 70 µg of DNA from leukemia N57 was digested to
completion with *Bam*HI and fractionated by electrophoresis in TAE
buffer. Fragments approximating the somatically acquired pAKV5
(ecotropic murine leukemia virus) hybridizing fragments were cut
from the gel and purified using Qiaex beads according to the
20 manufacturer's instructions (Qiagen, Valencia, CA). The *Bam*HI

fragments were cloned into EMBL4 phage arms (Stratagene, La Jolla, CA). Ligated material was packaged with Gigapack Gold packaging extracts (Stratagene, La Jolla, CA). Library screening was performed according to the protocol of Benton and Davis (1977). The P1 and 5 lambda genomic clones were derived from commercial libraries (Genome Systems, St. Louis, MO, Clontech, Palo Alto, CA).

EXAMPLE 4

10 **Human Chromosome Mapping**

Metaphase chromosomes from normal peripheral blood samples were prepared using conventional methods. Slides were incubated in 2XSSC (pH 7.0, 37°C) for 15 minutes then immersed in 0.1N HCl/0.05% Triton X-100 for 15 minutes at room temperature 15 (RT). Slides were washed in 2XSSC (pH 7.0) twice at room temperature, then washed in 1XPBS, pH 7.2 once at room temperature. Slides were immersed in 1% formaldehyde (diluted in PBS) for 10 minutes at room temperature, washed twice in 1XPBS at room temperature, then once in 2XSSC (pH 7.0) at room 20 temperature. Chromosomes were dehydrated in an ethanol series

and air dried at room temperature.

A human PAC clone specific for *EVI27* was isolated by screening a human P1 artificial chromosome (PAC) library according to the manufacturer's instructions and high molecular weight PAC DNA was isolated using the KB-100 kit (Genome Systems, St. Louis, MO). Spectrum-Red-dUTP (Vysis, Downer's Grove, IL) was incorporated into PAC/BAC probes by nick translation according to the manufacturer's protocol (Vysis, Downer's Grove, IL). DNA was resuspended in Hybrisol VII (Oncor, Gaithersburg, MD) (50% formamide/2xSSC) at a final concentration of 5 ng/ul and hybridized to chromosomes according to manufacturer's instructions. The chromosomes and probe solution was incubated at 75°C for 10 minutes. then to 37°C for 16 hrs in a humidified chamber. Post hybridization washes consisted of 65% formamide /2xSSC (pH 7.0) for 15 minutes at 43°C, then 2XSSC (pH 7.0) for 8 minutes at 37°C, 1XPBD (Oncor, Gaithersburg MD) twice at room temperature, then in 1XPBS (pH 7.2). Finally, 10 µl of propidium iodide/antifade (1:20) was added on each area and a coverslip added. Following FISH, the slide was washed twice in Xylene to remove the coverslip and excess oil. The slide was then washed in

1XPBD at 37°C for 5 minutes followed by a dehydration step through an ethanol series. The slide was then incubated in 50% formamide/2XSSC for 90 minutes at 37°C, washed briefly in tap water, followed by dehydration in 10, 80, and 95% ethanol and 5 stained with Wright's stain/phosphate buffer for 5 minutes.

EXAMPLE 5

Exon Trapping

The P1 clone p57P1 was digested to completion with *Bgl*II and *Bam*HI and purified by phenol extractions and ethanol precipitations. Insert DNA was then ligated to a *Bam*HI digested pSPL3 vector (Life Technologies, Gaithersburg, MD). DNA from the ligations was used to transform DH10B maximum efficiency cells (Life Technologies, Gaithersburg, MD). Additional steps in the experiment were performed exactly according to the manufacturers protocol (Life Technologies). DNA sequence of approximately 100 individual clones from each ligation was generated as described below.

EXAMPLE 6

Poly(A)+ RNA Isolation and Northern Blot Analysis

Premade Northern blots containing 2 µg of twice selected poly(A)+ RNA from various normal tissues and cell lines were purchased from commercial source (CLONTECH, Palo Alto, CA). Total RNA was extracted from cell line suspensions by the RNazol B method (Tel-Test, Friendswood, TX). poly(A)+ RNA was purified from the total RNA preps by oligo-d(T) column chromatography according the manufacturers recommendations (Amersham Pharmacia Biotech, Piscataway, NJ). 2-5 µg of poly(A)+ RNA was fractionated by electrophoresis in 1.0 % agarose gels containing formaldehyde and transferred to Hybond N+ membranes (Amersham Pharmacia Biotech, Piscataway, NJ). The membranes were prehybridized and hybridized according to the method of Church and Gilbert or using ExpressHyb solution (Clontech, Palo Alto, CA). Blots were then exposed to X-ray film at -70°C with an intensifying screen.

EXAMPLE 7

cDNA Cloning

cDNA cloning was carried out by a combination of 5' and 3' rapid amplification of cDNA ends (5' and 3' RACE) and modified 5 RT-PCR as described (Shaughnessy et al., 1999). Briefly, the nucleotide sequence of the trapped exon p57P1ET47 was used to design specific nested oligonucleotides. The primary 5' and 3' RACE reactions were performed using mouse liver Marathon Ready cDNA (Clontech, Palo Alto, CA) as a template according to the manufactures protocol using the Advantage cDNA amplification kit 10 (Clontech, Palo Alto, CA). After cloning and sequencing, nested primers specific for the 5' and 3' ends of the gene were synthesized. Another cycle of PCR was performed using Marathon Ready liver cDNA as a template and the full length cDNA was generated by PCR 15 as follows: 1 cycle: 94°C 1min; 30 cycles: 94°C, 30 sec, 68°C, 7 min. The products of the reaction were subcloned into pCR2.1 (Invitrogen, Carlsbad, CA) and sequenced. The full-length cDNA of the 1.9 kb and 2.7 kb transcripts have been submitted to GenBank under accession numbers AF208108 and AF208109, respectively.

Human cDNAs covering the coding region of the *EVI27* gene were synthesized in a similar manner as described above. Briefly, a human EST (accession number T96740), with high homology to the mouse *EVI27* cDNA, was used to design nested 5 oligonucleotide primers at the 5' and 3' end of the EST. 5' and 3' RACE products from Marathon Ready fetal liver cDNA (Clontech, Palo Alto, CA) were synthesized as described above. The 1.9 kb and 2.9 kb human *EVI27* cDNA nucleotide sequences are deposited in GenBank under accession numbers AF208110 and AF208111.

EXAMPLE 8

Transfection and Stable Cell Lines

15 The 1.9 kb mouse *Evi27* cDNA was cloned into the *NotI*-
HindIII site of the pcDNA3.1(-) eukaryotic expression vector and
transformed into DH5 α bacteria. 10 μ g of recombinant plasmid
DNA was resuspended at 1 μ g/ μ l in sterile TE. Cell lines 32Dcl3,
M1,WEHI3B, and NIH3T3 were grown in appropriate media
20 maintaining cell viability at 90%. Cells were harvested and washed

3X in FBS-free DMEM media. Cells were resuspended at 5×10^6 cells per ml in FBS-free DMEM. One ml of the cell suspension was mixed with DEAE dextran at 0.1 $\mu\text{g}/\text{ml}$ and 10 μg of plasmid DNA in an 0.4 cm electrode gap electroporation cuvette and chilled on ice for 10 minutes and Eletroporated at 270 V/975 μF in a Gene Pulser II (Bio-Rad, Hercules, CA). The cuvette was chilled on ice for 10 minutes then the cells were transferred into 10 ml of fresh media and grown for 72 hrs. G418 selection was carried out in 2 mg/ml for 2 weeks. Limiting dilution cloning in 96 well culture dishes was performed at 1 mg/ml G418 on all cell lines with the exception of NIH3T3. Stable transfectants were cloned and tested for expression of the transgene by Northern blot hybridization.

EXAMPLE 9

15

Southern and Northern Blot Probes

The β -actin probe was a 2.0 kb cDNA (Clontech, Palo Alto, CA). Probes pAKV5 and pEco have been described (Nakamura et al., 1996). p57BXH2A was a 700 bp PCR fragment generated from

the flanking DNA from clone p57BXH2. Probe p57ET47 was a 103 bp exon trapped from p57 P1. The human intron probe was synthesized by PCR from genomic DNA. Human ESTs were derived from IMAGE consortium clones. Full length 1.9 kb human and mouse *Evi27* cDNAs were used in all probes were labeled with [α³²P]-dCTP using the Prime It II labeling kit (Stratagene, La Jolla, CA).

EXAMPLE 10

DNA Sequencing

DNA sequencing was performed using the PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit (Perkin Elmer) on the ABI Model 373A DNA Sequencer (Applied Biosystems).

Sequence primers were either the T3, T7 sequencing primers or synthetic oligomers derived from previously determined sequence.

EXAMPLE 11

Immunohistochemistry

Cells were harvested and washed once in PBS and
5 resuspended at 1-2 x 10⁶ cells per ml in PBS. 0.5 ml of the cell
suspension was subjected to cytocentrifugation at 1200 x g for 5-10
minutes and air dried for 15-20 minutes, rinsed in PBS and drained
well but not allowed to dry. Slides were fixed in fresh 4%
paraformaldehyde for 10 minutes followed by two washes in PBS.
10 Cells were permeabilized by incubation in acetone for 30 seconds at
RT. Slides were rinsed in PBS with four changes over 5 minutes.
Cells were blocked with 5% goat serum in PBS for 30 minutes at RT.
The polyclonal anti-peptide EVI27 antibody, 2954, was affinity
purified using the Sulfa-Link kit according to manufacturer's
15 instructions (Pierce, Rockford, IL). Antibodies were diluted in 1 M
HEPES buffer containing 0.15 N NaCl. Sixty microliters of the
primary antibody solution was added to slides and incubated for 30
minutes in a humidified chamber at RT. The primary antibody was
blocked with 100 µg of peptide by incubating for 1 hour at RT. This
20 solution was then added to the slides as above. After hybridization

the slides were washed three times for 5 minutes in 1XPBD (Oncor, Gaithersburg, MD). The secondary antibody, a rhodamine conjugated goat-anti-rabbit antibody (Pierce, Rockford, IL) was diluted in the same buffer as the primary antibody and the solution 5 was added to slides for 30 minutes. Slides were washed 3X in PBS for 5 minutes each wash. The nuclei were counterstained with DAPI at 1/40 dilution in antifade (Oncor, Gaithersburg, MD), coverslips added and viewed with an Olympus BX60 epifluorescence microscope.

EXAMPLE 12

Western Blotting

Cells were harvested at logarithmic growth stage and 15 washed 2X in 1XPBS. Cells were resuspended in extraction buffer (1x PBS (pH 7.2), 10 µg/ml Aprotinin (0.1 unit/ml), 10 µg/ml Leupeptin, 1mM PMSF) at 10^6 cells/100 µl. Protein was extracted by the freeze-thaw method. The solid phase was removed by centrifugation at 12,000g x 10 minutes at 4°C. The aqueous phase

was collected concentrated and quantified using the BCA assay kit (Pierce, Rockford, IL). Protein separating gels were precast (Novex, San Diego, CA). Protein (50-100 µg) was mixed 1:1 with sample buffer (2X) (Novex, San Diego, CA), denatured for 15 minutes, 5 cooled to RT and loaded. Gels were run for 2-3 hours at 200V with cooling (60W initially) in 1X running buffer (25 mM Tris, 192 mM glycine, 0.1% SDS at pH 8.3). Protein was transferred to Hybond-C super membrane (Amersham Pharmacia Biotech, Piscataway, NJ) using an electrophoretic blotting system (C.B.S. Scientific Co. Del Mar, CA) in 1X transfer buffer (48mM Tris, 39 mM glycine, 20% methanol) at 100V for 5-6 hrs. The western blot was processed as described (WesternBreeze kit, Novex, San Diego, CA).

15

EXAMPLE 13

Computer DNA and Protein Sequence Analysis

Sequence homology searches were conducted at the protein level using the National Center for Biotechnology 20 Information and the BLAST network service. The DNA and protein

sequence alignments and protein motif sequence searches were performed using MacVector (Oxford Molecular Group, Beaverton, OR) and pSORT (Nakai and Kanehisa, 1992).

5

EXAMPLE 14

Cloning and Characterization of Genomic Sequences from the *Evi27* locus

Genomic restriction analysis of DNA from two BXH2 leukemias with proviral integrations at *Evi27* (N57 [B160] and 15-38374) showed that the integrations in the two leukemias are located about 1.5 kb apart and are oriented in the same transcriptional direction (Figure 1). Lambda and P1 clones from the region were then isolated, restriction mapped and exon trapped. Four exon-trapped products were identified. BLAST searches showed that two of the exons were derived from the mouse choline dehydrogenase (*Chdh*) gene, while one exon was derived from a gene with high homology to the yeast actin-like protein, ARP8 (accession number S67026). The *Chdh* gene was subsequently

positioned ~10 kb to the left of the proviral integration sites and in the opposite transcriptional direction (Figure 1). Northern analysis showed that *Chdh* expression is restricted to liver and is not activated in the B160 cell line (a cell line derived from the N57 5 leukemia that carries a proviral integration at *Evi27*). The *ARP8* homologue was mapped ~15-20 kb to the right of the proviral integration sites. Northern analysis showed that this gene is ubiquitously expressed and is not upregulated in B160 leukemia cells. On this basis, *Chdh* and *ARP8* were excluded as candidate disease genes.

The fourth exon (p57ET47) showed no significant homology to any sequences in GeneBank. Hybridization studies showed that this exon was contained within lambda clone p57λ129 (Figure 1), indicating that it was located within 5-20 kb of the 15 proviral integration sites at *Evi27*. DNA sequence analysis of p57λ129 identified a 103 bp fragment that was homologous to a human fetal liver cDNA EST (accession number T96740). This EST sequence was localized 1240 bp upstream of p57ET47 and was found to be oriented in the same transcriptional direction, 20 suggesting that both exons might be derived from the same gene.

Northern blot analysis of mouse tissues using both exons as probes confirmed this prediction and showed that the *Evi27* hybridization pattern is complex. Six different *Evi27* transcripts of approximately 4.4, 4.2, 2.3, 1.9, 1.3, and 1.1kb in size, could be
5 detected on northern blots (Figure 2). In adult tissues the expression was seen in liver and testes where the 2.3 and 1.9 kb transcripts predominated. Expression of the 4.2 transcript was also seen in liver and testes. In addition, low levels of the 2.3 and 1.9kb transcripts were seen in kidney (Figure 2A). With long exposure, the
10 lung showed expression of a 2.1kb transcript, while the heart showed expression of the 1.3kb transcript. No expression was seen in skeletal muscle, brain, or spleen.

In addition to adult tissues, *Evi27* expression was also detected in some hematopoietic cell lines. These cell lines include
15 the EL-4 T-cell line, which express the 4.2 and 1.3 kb transcripts, the WEHI231 B-cell line which expresses the 4.2, 1.3 and 1.1 kb transcripts, and the R1.1 T-cell line, which expresses the 1.9 kb transcript. In contrast, little or no expression was detected in the 32D, WEHI 3B, and M1 myeloid cell lines, the HYB.548 B-cell line, or
20 the P815 mast cell line (Figure 2C).

Evi27 expression could also be detected in BXH2 myeloid cell lines whether or not they carry a proviral integration at *Evi27*. Expression of the 4.4, 4.2, 1.9 and 1.1kb transcripts was variable among the different lines (Figure 2C), but the highest expression was 5 seen in the B160 cell line, which contains a viral integration at *Evi27*. In this cell line the 4.4 kb transcript predominated. These results suggest that viral integration at *Evi27* results in increased *Evi27* expression.

In humans, two *EVI27* transcripts, 1.9 kb and 2.7 kb in size, were detected (Figure 3). Human *EVI27* expression is therefore considerably less complex than in mouse. The highest *EVI27* expression was seen in kidney (Figure 3A), while in the mouse, *Evi27* was expressed at low levels in the kidney. Moderate expression was also observed in the brain, liver, and testes with low to undetectable 15 expression in lung. No *EVI27* expression was seen in heart, placenta, and skeletal muscle (Figure 3A) or in immune tissues such as spleen, lymph nodes, thymus, peripheral blood lymphocytes, or bone marrow (Figure 3B). High *EVI27* expression was, however, observed in the fetal liver.

20 Within cell lines, moderate *EVI27* expression was seen in

the cervical carcinoma HeLa, chronic myelogenous leukemia, K562, and colon adenocarcinoma, SW480 cell lines (Figure 3C), but not in the myeloid leukemia HL-60, acute lymphoblastic T-cell leukemia MOLT-4, Burkitt's lymphoma Raji, lung carcinoma A549 and the 5 melanoma G361 cell lines.

EXAMPLE 15

10 **The Gene Whose Expression is Upregulated by Viral integration at Evi27 encodes a Novel IL-17 Receptor-Related Protein**

15 The complete coding region of human gene was obtained by 3', and then 5' RACE. The primary 5' RACE product using primers from the 3' end of the gene was ~1.9 kb in length, consistent with the size of the major human transcript. A larger minor species was also produced in the 5' RACE reaction. Sequence analysis showed that it contained an additional 950 bp of sequence that was not present in the 1.9 kb product. PCR and genomic sequence analysis showed that this extra sequence was an unspliced intron. Northern 20 analysis showed that the 2.7 kb human transcript contains this

unspliced intron and sequence analysis showed that this unspliced intron introduces multiple stop codons into the open reading frame of the protein.

The sequence of the full length 1.9 kb and 2.7 kb transcripts has been deposited in GenBank with accession numbers AF208110 and AF208111, respectively; SEQ. ID NO: 1 and SEQ. ID NO: 2 respectively in the present invention. The predicted uninterrupted protein is 502 amino acids (aa) and has a predicted molecular weight of 56 kD (Figure 4). pSORT analysis identified a potential cleavable signal peptide at the N terminus of the protein. A putative transmembrane domain starts at residue 293 and ends at residue 309. The cytoplasmic tail is predicted to extend from residue 310 to the C terminus. pSORT analysis also indicated that the protein has type 1a topology. A string of 39 amino acids derived from intronic sequences would be inserted at amino acid 250 in the 2.7 kb transcript. This would result in the synthesis of a 288 amino acids protein with a predicted molecular weight of 31 kD. The transmembrane and cytoplasmic portions of the 502 amino acids isoform are missing in this truncated form of the protein.

Multiple 5' RACE products were also obtained in mouse.

Cloning and sequence analysis of these RACE products showed that they can be created by the inclusion of at least two unspliced introns. As in human, these intron sequences introduce multiple stop codons into the open reading frame of the protein. The 5 sequence of the full length 1.9 kb and 2.5 kb mouse transcripts has been deposited in GenBank with accession numbers AF208108 and AF208109, respectively; SEQ. ID NO: 3 and SEQ. ID NO: 4 respectively in the present invention. The mouse 4.4, 4.2, 2.4 and 1.3 kb transcripts have not been cloned and sequenced.

10 Genomic sequence analysis indicates that the mouse gene consists of 11 exons and spans ~13 kb of genomic DNA (Figure 5). It is interesting to note that Evi27 cDNAs specifically lacking exon 10 have been cloned from mouse AML cell lines. The gene is transcribed in the same orientation as the integrated proviruses, 15 which lie in a CpG rich region located approximately 6 kb 5' from the first known exon of the gene (Figure 1). The predicted open reading frame of the 1.9 kb transcript is 499 amino acids with a predicted molecular weight of 56 kD (Figure 4). pSORT analysis identified a potential cleavable signal peptide in the N terminus of 20 the protein, just like in the human protein. A putative

transmembrane domain starts at amino acid 290 and ends at amino acid 293. The transmembrane domain is located in exon 12 of the gene and is spliced out in variant transcripts sequenced from the WEHI-231 B-cell line. These variant transcripts splice out an exon 5 containing the entire putative transmembrane domain. The lack of this exon does not disrupt the predicted open reading frame of the protein.

In the 2.5 kb transcript, a string of 57 novel amino acids, derived from intron 7, is added at amino acid 162 creating a protein with an open reading frame of 218 amino acids and a predicted molecular weight of 24 kD. Like the shorter human isoform, this isoform would lack the transmembrane and cytoplasmic domains of the protein.

The full-length human and mouse proteins are 83% similar and 76% identical (Figure 4). The extracellular domain of both proteins contains three conserved N-linked glycosylation and a GSK3 phosphorylation site. A single conserved GSK3 phosphorylation site is also present in the cytoplasmic domain.

Amino acid sequence comparisons showed that *Evi27* has 20 significant homology throughout its coding region to the human and

mouse *IL-17 receptor* (*IL-17R*) (E value = 2.5 e-29) (Figure 6). The position of the transmembrane domain with respect to the amino terminus is essentially the same in the two proteins. However, the cytoplasmic tail of the *IL-17R* is nearly 304 amino acids longer than that of the *Evi27*. Although *Evi27* is predicted to encode both membrane bound and soluble forms, *IL-17R* is not known to encode a soluble form.

EXAMPLE 16

The Mouse and Human Genes map in Syntenic Regions

The human *EVI27* gene was mapped to chromosome 3p21 by fluorescence *in situ* hybridization of high-resolution G-banded chromosomes (Figure 7). These results are consistent with the mouse mapping data, which localize the mouse gene to chromosome 14 in a region of human 3p21 homology (Copeland et al., 1995). They also confirm that the human gene maps in a region of the human genome that is frequently rearranged in human myeloid leukemia.

EXAMPLE 17

Expression and Subcellular Localization of Evi27

The expression and subcellular localization of the mouse
5 protein was examined using a polyclonal antibody raised against a
peptide derived from the C-terminus of Evi27. Antibody specificity
was confirmed by immunofluorescence staining of 32D cells, which
express undetectable levels of *Evi27* mRNA (Figure 8A), and 32D
cells stably transfected with a 2.7 kb *Evi27* cDNA clone. Western
blot analysis showed that whereas the parental 32D cells show weak
expression of the expected 55 kD full length protein, the transfected
line expresses approximately 3-5 fold higher levels of this isoform
(Figure 8B). Immunofluorescence analysis showed weak staining in
the parental 32D cells whereas the transfected cells show very
15 abundant staining on the surface with a capping pattern (Figure 8C,
left panels). Surface expression of Evi27 was further shown by flow
activated cell sorting analysis (Figure 8D). Clear cell surface
staining was observed after analysis of live cells or fixed, but not
permeabilized, transfected cells (data not shown). In all
20 experiments the staining could be quenched by preincubation of the

antisera with Evi27 peptide (Figure 8C, right panels and Figure 8D), thus indicating the antiserum is specific for Evi27.

Murine T cell lymphoma EL-4 cells also express Evi27 on cell surface and the cell surface capping possibly indicates 5 polydimerization and receptor activation of Evi27 (Figure 9). In contrast, Evi27 shows different localization in murine AML cells. Myeloid leukemia cells B187 and B160 both show diffuse speckled staining pattern in the cytoplasm, whereas the B190 cells show a capping pattern similar to the T cell lymphoma EL-4 (Figures 10-11). Moreover, abundant Evi27 protein was found in the cytoplasm, but not on the cell surface, of the B160 cells (Figure 11).

EXAMPLE 18

15

Western blot analysis of Evi27 Protein Expression

Western blot analysis of a number of leukemia cell lines confirmed the Northern results and showed that many different Evi27 isoforms are expressed at the protein level (Figure 12). A 56 20 kD protein, which is the predicted size of the full length protein

containing the transmembrane and cytoplasmic domains, was expressed in most samples tested (Figure 12A). The EL4 and R1.1 T-cell lines and the WEHI 231 B-cell line also express a 24 kD protein, which is the predicted size of the truncated protein resulting from 5 intron incorporation and an 85 kD protein (Figure 12A). This protein is also expressed at low levels in the B190 cell line but not in the other the BXH2 leukemia cell lines tested.

Several cytokine receptors also produce soluble forms of the receptor by proteolytic cleavage of the membrane bound receptor. The cleavage sites are located near the plasma membrane in the extracellular domain of the receptor. Proteolytic cleavage of the Evi27 membrane receptor in this region is predicted to produce a 33 kD protein. A band of this size is also present in most samples tested (Figure 12A). Several unexpected sized Evi27 bands were also 15 detected on the Western blot. These protein bands can all be competed away by the addition of Evi27 peptide (Figure 12B), indicating that they contain authentic Evi27 protein sequences.

The origin of these protein bands is unknown but could result from the translation of Evi27 mRNA transcripts that have yet 20 to be sequenced or may be indicative of homo- or heteropolymer

formation. The expression of these protein bands is sometimes variable. For example, the 75 kD band is expressed at low or undetectable levels in the B160 and B187 cell lines. The B193 cell lines does not express the 150 kD and 127 kD bands, but does 5 expresses high levels of the 75 and 33 kD bands. The reason for this is not clear, but may reflect the differentiation state of the cell line. Consistent with this hypothesis, T- and B-cell lines express Evi27 protein bands that are not expressed in BXH2 myeloid cell lines (i.e., 24 and 85 kD bands).

Evi27 expression was also examined in human hematopoietic cells. Western blot analysis showed that MEG01 (megakaryocytic leukemia), K562 (chronic myelogenous leukemia) and HEL (erythrocytic leukemia) cell lines express the highest levels of Evi27 protein (Figure 13). Flow activated cell sorting analysis 15 indicated that there is high level of surface expression on THP-1 (monocytic leukemia) and K562 cells but not on HL-60 (promyelocytic leukemia) cells (Figure 14).

Discussion

The studies described here show that retroviral integration at the *Evi27* locus in BXH2 murine myeloid leukemias results in the increased expression of a novel gene with homology to the *IL-17 receptor*. A number of cytokine receptors (i.e., *v-mpl* or *Tpor*, *Il6ra*, *Epor*, *Il9r*, *Csf1*, *Il2ra*, *Il4ra*, and *Ifngr2*) or their ligands (i.e., *Il6*, *Csf1*, *Il3*) are reported targets of retroviral integration or transduction in mouse leukemia/lymphomas making cytokines/cytokine receptors one of the largest and most important classes of leukemia genes (Ymer et al., 1985; Wendling et al., 1986; Penciolelli et al., 1987; Gisselbrecht et al., 1987; Sugita et al., 1990; Blankenstein et al., 1990; Souyri et al., 1990; Hino et al., 1991; Chretian et al., 1994; Flubacher et al., 1994; Li et al., 1999). The *Evi27*-encoded cytokine-related receptor is therefore an excellent candidate for a new leukemia disease gene.

The human *EVI27* gene was also cloned and mapped to chromosome 3p14-p21, in a region syntenic to the mouse gene. The human protein is 76% identical to the mouse protein at the amino acid level. This is somewhat higher than the recently described homology between the mouse and human IL-17 receptors, which are

69% identical at the amino acid level (Yao et al., 1997). This conservation suggests that *Evi27* receptor function is evolutionarily conserved between human and mouse.

Proviral integrations at *Evi27* are located about 6 kb upstream from the *IL-17 receptor*-related gene and result in increased gene expression. Viral integration at *Evi27* may thus induce disease simply by upregulating receptor expression. However, given the complex expression pattern observed for *Evi27*, it is also possible the viral integration induces disease by altering *Evi27* isoform expression. In the mouse, as many as six *Evi27* transcripts are detected on Northern blots. Two of the transcripts result from intron incorporation and are predicted to produce a truncated soluble form of the receptor lacking the transmembrane and cytoplasmic domains. Several cytokine receptors are known to produce soluble as well as membrane bound forms and it is now well documented that the soluble receptors can have both positive and negative effects on ligand signaling (for review see Heaney and Golde, 1996). The origin of the other *Evi27* transcripts has not been determined. It is thus possible that one or more of these uncharacterized messages encode sequences that are located

upstream of *Evi27* proviral integration sites at *Evi27*. If this is the case, then *Evi27* proviral integrations may prevent these messages from being expressed and this may have important disease consequences.

5 *IL-17r /Evi27* are unusual in that they have no homology with any protein in public databases, including other cytokine receptor proteins, and they have no recognizable motifs associated with intracellular signalling (Yao et al., 1995b). Many disease-related cytokine receptors involved in leukemogenesis contain kinase domains; however, it is not unprecedented to find receptors lacking kinase domains that are involved in disease. For example, the human *MAS1* oncogene, which encodes a functional angiotensin receptor, is a G-protein coupled receptor that functions through phosphatidylinositol 4,5-bisphosphate hydrolysis (Young et al., 15 1986; Jackson et al., 1988). *Evi27* may thus identify a new disease pathway.

At least seven different *Evi27* isoforms (i.e., 24, 33, 56, 47, 75, 127, and 150 kD in size) were also detected in BXH2 leukemia cell extracts by western analysis using an *Evi27*-specific 20 antibody. All leukemia extracts expressed the 56 kD isoform (the

membrane bound receptor) as well as the 33 kD isoform (a postulated proteolytic cleavage containing the extracellular ligand-binding domain of the receptor). Expression of the other isoforms was variable among the extracts. One of seven cell extracts (B190) 5 expressed the 24 kD isoform (a putative truncated receptor resulting from intron incorporation), three extracts (B139, B160, and B187) express the 47 kD isoform, five extracts (B112, B132, B139, B190, B193) the 75 kD isoform, and six extracts (B112, B132, B139, B160, B187, B190) the 150 kD isoform. The origin of these 10 larger isoforms (i.e., oligomerization) or the reason for their variable expression (i.e. differentiation state of the cell) is presently unclear.

Only one BXH2 leukemia cell extract, B160, analyzed on Western blots is known to contain a proviral integration at *Evi27*.

15 The 56 kD isoform is overexpressed in B160 cells, consistent with Northern results showing that viral integration at *Evi27* results in increased *Evi27* expression. Surprisingly, the 56 kD isoform is also overexpressed in B187 cells, which are not known to carry a viral integration at *Evi27*. Perhaps, B187 cells harbor a viral integration 20 at *Evi27* that maps outside the region examined on Southern blots or

there are other mechanisms for upregulating expression of this isoform other than proviral integration. It is interesting to note that B187 cells, like B160 cells, also fail to express the 75 kD isoform. One intriguing possibility is that viral integration at *Evi27* blocks 5 expression of this isoform.

Evi27 expression is restricted in the hematopoietic cells. Northern analysis showed that *Evi27* is expressed in T cells and ESTs homologous to the mouse gene have been identified in CD4⁺ T-cells. Likewise, the mouse preB-cell line WEHI231 expresses *Evi27* and ESTs homologous to *EVI27* have been identified in human germinal center 10 B cells. No *Evi27* expression was seen in the late stage B-cell line 548, the Burkitt lymphoma cell line Raji, the murine erythroid line D1b, or the mast cell line P815. A complex pattern of expression 15 was observed within the myeloid compartment. Variable expression was seen in BXH2 leukemia cells, while little or no *Evi27* expression was seen in the M1 and 32D murine myeloid leukemia cell lines or in the monocytic leukemia cell line WEHI-3B. *Evi27* is expressed, however, in the chronic human myelogenous leukemia cell line K562, but not in the promyelocytic leukemia cell line HL-60. These 20 results suggest that *Evi27* expression may be tightly regulated during

myeloid cell differentiation and imply that *Evi27* may have an important function in controlling the growth and/or differentiation of hematopoietic cells. Proviral integration at *Evi27* may interfere with this function(s) and, in doing so, lead to myeloid disease.

5 The *Evi27* subcellular protein distribution was also analyzed in various mouse hematopoietic cell lines. In the EL4 T-cell and WEHI231 B-cell lines, *Evi27* protein was largely found on the cell surface in a capping pattern. Interestingly, only one BXH2 leukemia cell line, B190, has a similar staining pattern. In the other BXH2 leukemia cell lines examined, including B160, which has a proviral integration at *Evi27*, *Evi27* protein is distributed throughout the cytoplasmic where it exhibits a punctate staining pattern. Western blot analysis shows that cells exhibiting the ER/Golgi staining pattern uniquely express the 24 kD *Evi27* isoform. It is possible that the 24
10 kD isoform uniquely localizes to the ER/Golgi and somehow prevents the other *Evi27* isoforms from entering the cytoplasm. Alternatively, it is possible that a cofactor or heterodimeric partner required for transport from the ER/Golgi complex is differentially expressed. This hypothesis is strengthened by the fact that the 32D
15 cell line transfected with the 56kD isoform still lacks the
20

cytoplasmic distribution seen in B160 or B187. In support of this possible mechanism, the expression of beta-2-microglobulin is required for cell surface expression of MHC class I or class I-like molecules (Lamouse-Smith et al., 1993; Feder et al., 1998).

5 Additionally, the receptor-associated protein (RAP), a type of chaperone, is especially designed to assist in the biosynthesis and intracellular transport of endocytic receptors (Willnow, 1998) and band 3 (AE1 gene) plays a chaperone-like role required for the recruitment of Glycophorin A to the red blood cell plasma membrane (Hassoun et al., 1998). Finally, recent studies have shown that calnexin and Ig-alpha/Ig-beta interactions with membrane immunoglobulins are critical for the surface expression of the B cell antigen receptor of the IgM and IgD classes (Wu et al., 1997).

15 IL-17 (CTLA8) is a homodimeric cytokine of about 32 kD expressed exclusively from human memory T cells or mouse alpha beta TCR⁺CD4⁻CD8⁻ thymocytes (Rouvier et al., 1993; Yao et al 1995a; Yao et al., 1995b; Kennedy et al., 1996). In contrast with the tightly controlled expression of the ligand, the IL-17 receptor is 20 ubiquitously distributed but more abundant in spleen and kidney

(Yao et al., 1995b). Although devoid of direct effects on cells of hematopoietic origin, IL-17 induces the secretion of IL-6, IL-8, PGE2, MCP1 and G-CSF by adherent cells like fibroblasts, keratinocytes, epithelial and endothelial cells (Yao et al., 1995a; Yao et al., 1995b; 5 Fossiez et al., 1996). When cultured in the presence of IL-17, fibroblasts can sustain the proliferation of CD34⁺ human progenitors and their preferential differentiation into neutrophils (Fossiez et al., 1996). Adenovirus-mediated transfer of murine IL-17 cDNA into liver has also been shown to induce a transient, but dramatic granulopoiesis *in vivo*, except in IL-6-deficient mice 10 (Schwarzenberger et al., 1998).

The Evi27 protein is a member of the IL-17 receptor family. Two new members of the IL-17 cytokine family have recently been identified and these studies have demonstrated that 15 IL-17B and IL-17C do not bind the IL-17 receptor extracellular domain, indicating that IL-17B and IL-17C are likely to bind unique, yet uncharacterized receptors (Li et al., 2000). In a survey of cytokine induction, IL-17B and IL-17C stimulate the release of TNF- α and IL-1 β from the monocytic leukemia cell line THP-1 (Li et al., 20 2000). RT-PCR analysis has shown that IL-17B is expressed by both

the B160 leukemia and the stromal cell on which it depends. It is speculated that Evi27 may represent the receptor for IL-17B or IL017C. Based on these data a model for Evi27 in myeloid leukemia development is proposed. Terminal differentiation of 5 myelomonocytic precursor cells likely result in the down regulation of Evi27 expression. However, proviral insertions at Evi27 result in constitutive expression of the receptor. Binding of IL-17B/C to the Evi27 receptor would trigger the release of TNF- α and the IL-1 β by the leukemic cells. The TNF- α and IL-1 β would in turn provoke the production of multilineage hematopoietic growth factors, adhesion molecules, and inflammatory cytokines by stromal cells (Bagby, 10 1994). These stromal cell derived factors then support the growth and survival of the leukemia cell and may account for the absolute dependence of the B160 leukemia on the stromal feeder layer for 15 dependence for growth and survival.

The human homologue of *Evi27* may also be involved in human disease. *EVI27* maps to chromosome 3p21, a region consistently deleted in a variety of human cancers. Loss of 3p heterozygosity is also frequently observed in renal cell carcinoma, 20 lung cancer and breast cancer and analysis of 3p allele loss in renal

cell cancer has localized a candidate tumor suppressor gene to 3p21 (van den Berg et al., 1997). These results are consistent with the hypothesis that chromosome 3p encodes a number of tumor suppressor genes. Given that *EVI27* maps to 3p21 and is expressed at high levels in normal human kidney, it will be interesting to determine whether this gene is affected by 3p21 mutations in renal cell carcinoma. *EVI27* also may be a human myeloid leukemia disease gene. Recurrent treatment-related chromosome 3p21 breaks are frequently observed in myelodysplastic syndrome and acute myeloid leukemia patients (Shi et al., 1996), and 3p21 is the most frequently deleted region in human CML (Johansson et al., 1997). Future studies will be aimed at determining whether *EVI27* is a human myeloid leukemia disease gene.

An Evi27 ligand, IL-17E, that induces activation of NF- κ B and stimulates production of the proinflammatory chemokine IL-8 was identified recently. Together with the restricted expression of Evi27 disclosed herein, it is likely that Evi27 mediates the secretion of proinflammatory cytokines such as IL-8 and plays important role in the developmental and/or disease processes of hematopoietic cells. Hence, modulating the expression of Evi27 at the RNA or

protein level may be exploited for use in the treatment of diseases such as cancer or autoimmune diseases. A number of methods can be used to modulate Evi27 expression, e.g. by anti-sense oligonucleotides, small molecules that bind to the receptor,
5 modified IL-17E ligand that may stimulate or repress the activity of the Evi27 receptor, and the use of soluble form of the Evi27 receptor.

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Any patents or publications mentioned in this specification are indicative of the levels of those skilled in the art to which the invention pertains. Further, these patents and publications are incorporated by reference herein to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

One skilled in the art will appreciate readily that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those objects, ends and advantages inherent herein. The present examples, along with the methods, procedures, treatments, molecules, and specific compounds described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention as defined by the scope of the claims.